

## Embryonic stem cell-derived endothelial cells engraft into the ischemic hindlimb and restore perfusion.

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### Public Summary:

**OBJECTIVE:** We examined the effect of delivery modality on the survival, localization, and functional effects of exogenously administered embryonic stem cells (ESCs) or endothelial cells derived from them (ESC-ECs) in the ischemic hindlimb. **METHODS AND RESULTS:** Murine ESCs or ESC-ECs were stably transduced with a construct for bioluminescence imaging (BLI) and fluorescent detection. In a syngeneic murine model of limb ischemia, ESCs or ESC-ECs were delivered by intramuscular (IM), intrafemoral artery (IA), or intrafemoral vein injections (n=5 in each group). For 2 weeks, cell survival and localization were tracked by BLI and confirmed by immunohistochemistry, and functional improvement was assessed by laser Doppler perfusion. BLI showed that ESCs localized to the ischemic limb after IM or IA, but not after intrafemoral vein administration. Regardless of the route of administration, ESCs were detected outside the hindlimb circulation in the spleen or lungs. ESCs did not improve limb perfusion and generated teratomas. In contrast, ESC-ECs delivered by all 3 modalities localized to the ischemic limb, as assessed by BLI. Most surprisingly, ESC-EC injected intrafemoral vein eventually localized to the ischemic limb after initially lodging in the pulmonary circulation. Immunohistochemical studies confirmed the engraftment of ESC-ECs into the limb vasculature after 2 weeks. Notably, ESC-ECs were not detected in the spleen or lungs after 2 weeks, regardless of route of administration. Furthermore, ESC-ECs significantly improved limb perfusion and neovascularization compared with the parental ESCs or the vehicle control group. **CONCLUSION:** In contrast to parental ESCs, ESC-ECs preferentially localized in the ischemic hindlimb by IA, IM, and intrafemoral vein delivery. ESC-ECs engrafted into the ischemic microvasculature, enhanced neovascularization, and improved limb perfusion.

### Scientific Abstract:

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